Leica
BIOSYSTEMS

MAY 19 2014

1 510(k) Summary

Traditional Premarket Notification Submission 510(k) Summary
Prepared in accordance with 21 CFR 807.92

Assigned 510(k) number US K122556

2 Sponsor Name and Address

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4 Statement Prepared

July 8, 2012

5 Summary Revised:

April 15, 2014

6 Device Name

Trade (proprietary):	Estrogen Receptor Clone 6F11
Common (usual):	Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™
	Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™
Classification:	21 CFR § 864.1869: Immunohistochemistry (IHC)
	Reagents and Kits (Class II)
FDA Device Code:	MYA

Panel: 88 (Pathology)

7 Substantially Equivalent Predicate Device

Device Name: Estrogen Receptor Clone 6F11, formerly named Vision BioSystems Estrogen Receptor Clone 6F11 (ER 6F11) (change due to re-branding)

Device 510(k): K060227

8 Device Description

Estrogen Receptor Clone 6F11 is a mouse anti-human monoclonal antibody produced as a tissue culture supernatant. This antibody is utilized to perform a semi-quantitative immunohistochemical (IHC) assay to identify estrogen receptor (ER) expression in human breast cancer tissue routinely processed and paraffin-embedded for histological examination.

Estrogen Receptor Clone 6F11 primary antibody is provided in two formats, a Bond™ Ready-to-Use format (product code PA0151 (7mL) and PA0009 (30 mL)) and a concentrated liquid format (product code NCL-L-ER-6F11 (1mL) and is optimally diluted for use on the automated Bond System (Bond III) in combination with Bond Polymer Refine Detection kit.

Total protein concentration for Estrogen Receptor clone 6F11 is approximately 3.8 g/L. The immunoglobulin concentration is approximately 75 mg/L.

The Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ is recommended for use at a dilution of 1 in 50 when diluted in Bond Antibody Diluent (AR9352).

The Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ is a tissue culture supernatant prepared at a working immunoglobulin concentration of 0.88 µg/mL. It is supplied in Tris buffered saline with carrier protein, containing 0.35% ProClin™ 950 as a preservative.

9 Test Principle

Immunohistochemical staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody, ER), a secondary antibody to the primary antibody and an enzyme complex with a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

A schematic diagram of the immunohistochemical staining technique using a polymer detection system is shown in figure 1 and described below.

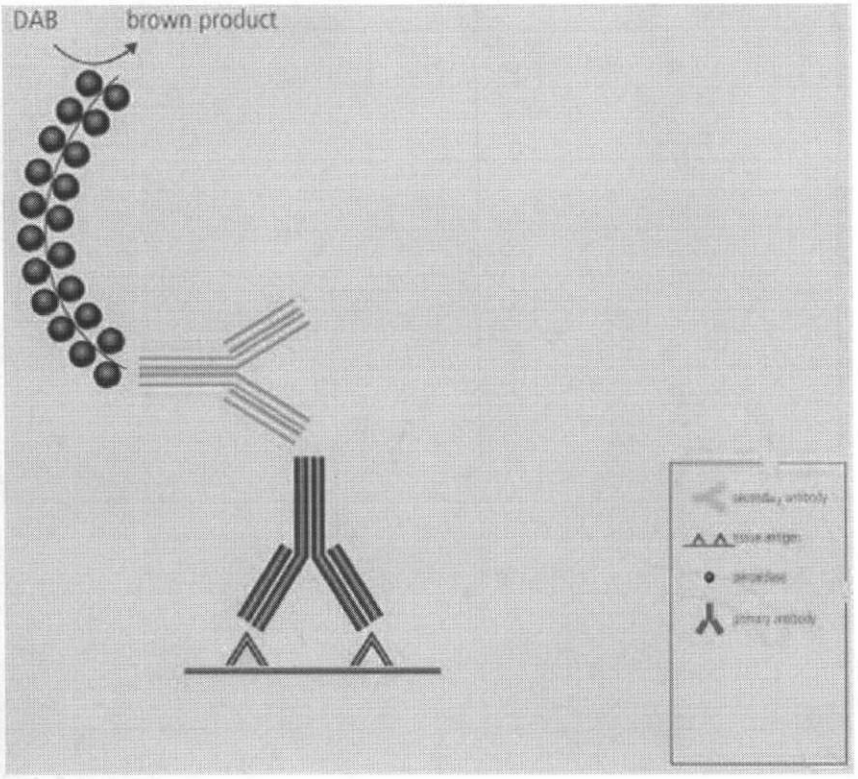

Figure 1. Schematic diagram of the immunohistochemical staining technique using a polymer detection system to detect the primary antibody.

Estrogen Receptor Clone 6F11 is recommended for use in an immunohistochemical procedure, which allows the qualitative identification by light microscopy of antigens in sections of formalin-fixed, paraffin-embedded tissue, via sequential steps with interposed washing steps. Prior to staining, endogenous peroxidase activity is blocked and sections are subjected to epitope retrieval. As indicated in figure 1, the section is subsequently incubated with the mouse primary antibody that binds with the human tissue antigen. A polymeric enzyme-conjugated secondary antibody that recognizes mouse immunoglobulins is used to detect the primary antibody. Sections are further incubated with the substrate/chromogen, 3,3' - diaminobenzidine (DAB), and DAB Substrate Buffer. Reaction with the peroxidase produces a visible brown precipitate at the antigen site. Sections are counterstained with hematoxylin and coverslipped. Results are interpreted using a light microscope.

10 Intended Use

For in vitro diagnostic use

Estrogen Receptor Clone 6F11 (ER 6F11) Mouse Monoclonal antibody is intended for laboratory use to qualitatively identify estrogen receptor (ER) antigen in sections of formalin fixed, paraffin embedded breast cancer tissue by immunohistochemistry method. Estrogen Receptor Clone 6F11 specifically binds to the ER antigen located in the nucleus of ER positive normal and neoplastic cells.

Estrogen Receptor Clone 6F11 is indicated as an aid in the management, prognosis and predication of therapy outcome of breast cancer. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ and Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ are optimized for use on the Leica Biosystems Bond III staining platform using the Bond Polymer Refine Detection kit.

11 Performance Characteristics (Clinical)

Clinical Outcome Study (Calgary Cohort)

The Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (PA0151) on the Bond III was tested in an independent clinical outcome study. In summary, the study used a retrospective Calgary-based patient cohort (n=532) composed of breast cancer patients diagnosed between 1985 and 2000, who were treated with primary adjuvant tamoxifen regardless of their ER and PR status. This cohort possesses several unique characteristics that lend to this study, including: it has greater than 5 years of follow-up; it was enriched for events to increase its statistical power; it contains ER negative patients so as to remove treatment selection bias.

To assess differences between methods in the study, the following statistical methods were evaluated and the outcomes described:

1. The Cohen's Kappa statistic to quantify the ease of reproducibility of Allred scoring method (Inter- and Intra-Observer).

Results indicated that Inter-observer kappa for the Leica platform showed almost perfect agreement for ER, with κ=0.67 between Observers 1 and 2, κ=0.75 between Observers 1 and 3, and a κ=0.83 between

Observers 2 and 3. Slides were also rescored by Observer 1 three months after the original scoring and intra-observer kappa was calculated with almost perfect agreement of κ=0.91.

2. Univariate Kaplan-Meier and Multivariate Cox survival analysis using the Allred cutpoint for hormone receptor positivity to dichotomize patients into survival groups.

The univariate outcome is shown in figure 2.

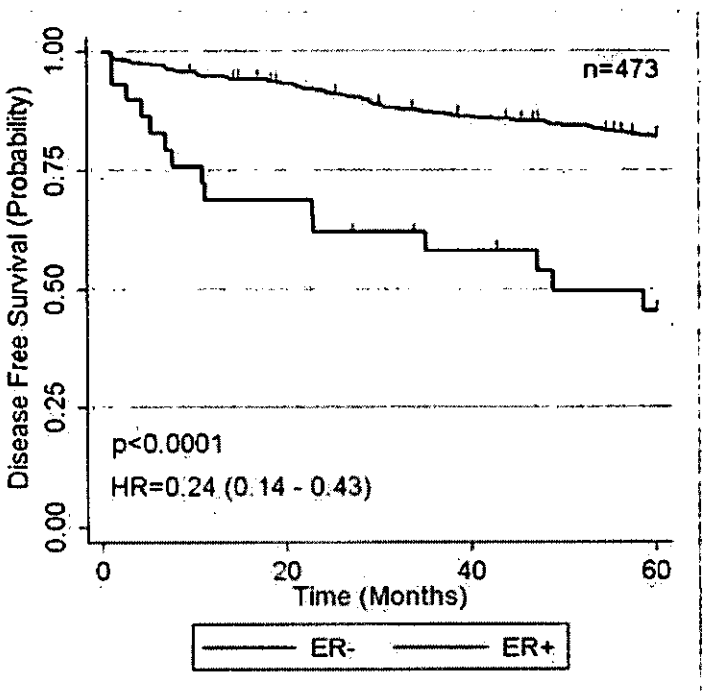

Figure 2: Univariate Analysis for the Leica test Device

Multivariate Cox models were analyzed along with lymph node status, tumour grade, tumour size and HER2 status. The model is shown in figure 3.

	Leica Device (n=363)		
	HR	95% CI	p-value
ER Status	0.39	(0.19 – 0.78)	0.008
Lymph Node Status	3.18	(1.88 – 5.37)	<0.001
Tumor Grade	3.15	(1.84 – 5.38)	<0.001
Tumor Size	1.67	(0.94 – 2.98)	0.083
HER2 Status	1.13	(0.38 – 3.33)	0.823

Figure 3: Multivariate model for the Leica test Device

3. Measures of test performance; sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), were calculated. For these calculations, the ligand-binding assay (LBA) was used as the gold standard (figure 4). Also, the Leica assay and the LBA using progression on tamoxifen as the gold standard (figure 5) were calculated.

In this study, each measured test performance can be defined as:

Sensitivity is defined as the proportion of positive subjects correctly identified by the test.

Specificity is defined as the proportion of negative subjects correctly identified by the test.

PPV is defined as the proportion of subjects with a positive test result who were correctly diagnosed.

NPV is defined as the proportion of subjects with a negative test result who were correctly diagnosed.

Figures 4 and 5 show the results obtained.

	Leica Device
Sensitivity	0.97
Specificity	0.44
PPV	0.96
NPV	0.70

Figure 4: Measure of test performance (LBA = Gold Standard)

	Leica Device	LBA
Sensitivity	0.96	0.96
Specificity	0.16	0.16
PPV	0.82	0.84
NPV	0.52	0.44

Figure 5: Measure of test performance (Progression = Gold Standard)

Results from this study show that the test device, the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (PA0151) on the Bond III has excellent correlation in identification of the optimal clinical assay for the determination of endocrine treatment response in breast cancer.

Additionally,

- The Leica platform used in the study was the Leica Bond III System.
- The test device used in the study was the PA0151 (Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™).
- The detection system used in the study was the Leica DS9800 Bond Polymer Refine Detection kit.

The staining protocol used in the study is as described below.

- The staining protocol used in the study was IHC Protocol F.
- Heat induced epitope retrieval using Bond Epitope Retrieval Solution 1 for 20 minutes was used.

- Bond Polymer Refine Detection utilizes a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates. The detection system avoids the use of streptavidin and biotin, and therefore eliminates nonspecific staining as a result of endogenous biotin. Bond Polymer Refine Detection works as follows:
- The specimen is incubated with hydrogen peroxide to quench endogenous peroxidase activity
- Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ is applied
- A post primary antibody solution enhances penetration of the subsequent polymer reagent
- A poly-HRP anti-mouse/rabbit IgG reagent localizes the primary antibody
- The substrate chromogen, 3,3'- diaminobenzidine (DAB), visualizes the complex via a brown precipitate
- Hematoxylin (blue) counterstaining allows the visualization of cell

All reagents and protocols used in this independent study were the same as that used in this 510K submission.

12 PRECISION RESULTS

Testing was initially conducted to assess within-run, within-instrument, between-run, between-laboratory and lot-to-lot precision using tissue micro-arrays (TMA) using the devices as described below.

12.1 Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the Bond III (Test Device)

Precision results for the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the Bond III are shown below.

12.1.1 Within Run Precision Study (intra assay – single instrument)

Testing was conducted on one SSA (1) [108 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (Lot 21825), 1 test slide with negative control antibody and 1 assay Control Slide] three times, over three different days.

		Positive	Negative
PA0151 (RTU) Bond III	Positive	81	0
	Negative	0	27
Overall Percent Agreement (95% CI)		100% (97.26-100.00)	
Positive Percent Agreement (95% CI)		100% (96.37-100)	
Negative Percent Agreement (95% CI)		100% (89.50-100)	

12.1.2 Within Instrument Precision Study (inter assay - single Instrument)

Testing was conducted on three SSA's (1, 2, 3) [321 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (Lot 21825), 3 test slides with negative control antibody and 3 assay Control Slide] three times, over three different days.

		Positive	Negative
PA0151 (RTU) Bond III	Positive	241	0
	Negative	0	80
Overall Percent Agreement (95% CI)		100% (99.07-100.00)	
Positive Percent Agreement (95% CI)		100% (98.76-100)	
Negative Percent Agreement (95% CI)		100% (96.32-100)	

12.1.3 Between Run Precision Study (inter assay - day-to-day - single instrument)

Testing was conducted on one SSA (1) [180 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (Lot 21825), 1 test slide with negative control antibody and 1 assay Control Slide] five times, over five different days, performed over a twenty (20) day period.

		Positive	Negative
PA0151 (RTU) Bond III	Positive	135	0
	Negative	0	45
Overall Percent Agreement (95% CI)		100% (98.35-100.00)	
Positive Percent Agreement (95% CI)		100% (97.81-100)	
Negative Percent Agreement (95% CI)		100% (93.56-100)	

12.1.4 Between Laboratory Precision Study (site-to-site - inter assay - multiple instruments)

Testing was conducted on one SSA (1) [101 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (Lot 21825), 1 test slide with negative control antibody and 1 assay Control Slide] at 3 investigational sites (Sites A, B and C).

		Positive	Negative
PA0151 (RTU) Bond III	Positive	78	0
	Negative	0	23
Overall Percent Agreement (95% CI)		100% (97.08-100.00)	
Positive Percent Agreement (95% CI)		100% (96.23-100)	
Negative Percent Agreement (95% CI)		100% (87.79-100)	

12.1.5 Lot to Lot Precision Study

Testing was conducted on one SSA (1) [100 test data points with Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™, 3 test slides with negative control antibody and 3 assay Control Slide] using three (3) independently manufactured reagent lots of the test device (Lot 1 = 21825; Lot 2 = 21866; Lot 3 = 21867).

		Positive	Negative
PA0151 (RTU) Bond III	Positive	75	0
	Negative	0	25
Overall Percent Agreement (95% CI)		100% (97.05-100.00)	
Positive Percent Agreement (95% CI)		100% (96.08-100)	
Negative Percent Agreement (95% CI)		100% (88.71-100)	

12.1.6 Between Observer Precision Study

Between observer precision testing was evaluated between 3 observers at 1 investigational site (Site A). Twenty (20) whole section breast cancer cases consisting of 5x Positive (Strong Intensity Expression) Profile Breast Carcinoma cases, 5x Positive (Medium Intensity Expression) Profile Breast Carcinoma cases, 5x Positive (Weak Intensity Expression) Profile Breast Carcinoma cases and 5x Negative Profile Breast Carcinoma were used.

Between observer agreement between Observer 1 and Observer 2 was 95% (19/20).

		Observer 1	
		Positive	Negative
Observer 2	Positive	14	0
	Negative	1	5
Overall Percent Agreement (95% CI)		95% (75.13-99.87)	
Positive Percent Agreement (95% CI)		93.33% (68.05-99.83)	
Negative Percent Agreement (95% CI)		100% (54.93-100)	

Between observer agreement between Observer 1 and Observer 3 was 95% (19/20).

		Observer 1	
		Positive	Negative
Observer 3	Positive	14	0
	Negative	1	5
Overall Percent Agreement (95% CI)		95% (75.13-99.87)	
Positive Percent Agreement (95% CI)		93.33% (68.05-99.83)	
Negative Percent Agreement (95% CI)		100% (54.93-100)	

Between observer agreement between Observer 2 and Observer 3 was 100% (20/20).

		Observer 2	
		Positive	Negative
Observer 3	Positive	14	0
	Negative	0	6
Overall Percent Agreement (95% CI)		100% (86.09-100)	
Positive Percent Agreement (95% CI)		100% (80.74-100)	
Negative Percent Agreement (95% CI)		100% (60.70-100)	

Between observer agreement between Observer 1, Observer 2 and Observer 3 was 98.33% (59/60).

		All Observers	
		Positive	Negative
All Observers	Positive	42	1
	Negative	0	17
Overall Percent Agreement (95% CI)		98.33% (91.06-99.96)	
Positive Percent Agreement (95% CI)		100% (93.12-100)	
Negative Percent Agreement (95% CI)		94.44% (72.71-99.86)	

12.2 Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ on the Bond III (Test Device)

Precision results for the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ on the Bond III are shown below.

12.2.1 Within Run Precision Study (intra assay – single instrument)

Testing was conducted on one SSA (1) [104 test data points with the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (Lot 6021831), 1 test slide with negative control antibody and 1 assay Control Slide] three times, over three different days.

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	79	0
	Negative	0	25
Overall Percent Agreement (95% CI)		100% (97.16-100.00)	
Positive Percent Agreement (95% CI)		100% (96.28-100)	
Negative Percent Agreement (95% CI)		100% (88.71-100)	

12.2.2 Within Instrument Precision Study (inter assay - single Instrument)

Testing was conducted on three SSA's (1, 2, 3) [313 test data points with the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (Lot 6021831), 3 test slides with negative control antibody and 3 assay Control Slide] three times, over three different days.

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	236	1
	Negative	0	76
Overall Percent Agreement (95% CI)		99.68% (98.23-99.99)	
Positive Percent Agreement (95% CI)		100% (98.74-100)	
Negative Percent Agreement (95% CI)		98.70% (92.96-99.97)	

12.2.3 Between Run Precision Study (inter assay - day-to-day - single instrument)

Testing was conducted on one SSA (1) [175 test data points with the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (Lot 6021831), 1 test slide with negative control antibody and 1 assay Control Slide] five times, over five different days, performed over a twenty (20) day period.

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	132	0
	Negative	0	43
Overall Percent Agreement (95% CI)		100% (98.30-100.00)	
Positive Percent Agreement (95% CI)		100% (97.76-100)	
Negative Percent Agreement (95% CI)		100% (93.27-100)	

12.2.4 Between Laboratory Precision Study (site-to-site - inter assay - multiple instruments)

Testing was conducted on one SSA (1) [104 test data points with the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (Lot 6021831), 1 test slide with negative control antibody and 1 assay Control Slide] at 3 investigational sites (Sites A, B and C).

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	78	0
	Negative	0	26
Overall Percent Agreement (95% CI)		100% (97.16-100.00)	
Positive Percent Agreement (95% CI)		100% (96.23-100)	
Negative Percent Agreement (95% CI)		100% (89.12-100)	

12.2.5 Lot to Lot Precision Study

Testing was conducted on one SSA (1) [107 test data points with the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™, 3 test slides with negative control antibody and 3 assay Control Slide] using three (3) independently manufactured reagent lots of the test device (Lot 1 = 6021831; Lot 2 = 6021836; Lot 3 = 6021857).

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	80	0
	Negative	0	27
Overall Percent Agreement (95% CI)		100% (97.24-100.00)	
Positive Percent Agreement (95% CI)		100% (96.32-100)	
Negative Percent Agreement (95% CI)		100% (89.50-100)	

12.2.6 Between Observer Precision Study

Between observer precision testing was evaluated between 3 observers at 1 investigational site (Site A). Twenty (20) whole section breast cancer cases consisting of 5x Positive (Strong Intensity Expression) Profile Breast Carcinoma cases, 5x Positive (Medium Intensity Expression) Profile Breast Carcinoma cases, 5x Positive (Weak Intensity Expression) Profile Breast Carcinoma cases and 5x Negative Profile Breast Carcinoma were used.

Between observer agreement between Observer 1 and Observer 2 was 89.47% (17/19). One case was omitted as it was reported by observer 1 as invasive tumour, while observer 2 reported

this as ductal carcinoma in-situ (DCIS) only, indicating a difference in the primary diagnosis of breast cancer.

		Observer 1	
		Positive	Negative
Observer 2	Positive	12	0
	Negative	2	5
Overall Percent Agreement (95% CI)		89.47% (66.86-98.70)	
Positive Percent Agreement (95% CI)		85.71% (57.19-98.22)	
Negative Percent Agreement (95% CI)		100% (54.93-100)	

Between observer agreement between Observer 1 and Observer 3 was 94.74% (18/19). One case was omitted as it was reported by observer 1 as invasive tumour, while observer 2 reported this as ductal carcinoma in-situ (DCIS) only, indicating a difference in the primary diagnosis of breast cancer.

		Observer 1	
		Positive	Negative
Observer 3	Positive	13	0
	Negative	1	5
Overall Percent Agreement (95% CI)		94.74% (73.97-99.87)	
Positive Percent Agreement (95% CI)		92.86% (66.13-99.82)	
Negative Percent Agreement (95% CI)		100% (54.93-100)	

Between observer agreement between Observer 2 and Observer 3 was 94.74% (18/19). One case was omitted as both observers reported a single case as ductal carcinoma in-situ (DCIS) only.

		Observer 2	
		Positive	Negative
Observer 3	Positive	12	1
	Negative	0	6
Overall Percent Agreement (95% CI)		94.74% (73.97-99.87)	
Positive Percent Agreement (95% CI)		100% (77.91-100)	
Negative Percent Agreement (95% CI)		85.71% (42.13-99.64)	

Between observer agreement between Observer 1, Observer 2 and Observer 3 was 96.49% (55/57). One case was omitted as it was reported by observer 1 as invasive tumour, while observer 2 and 3 reported this as ductal carcinoma in-situ (DCIS) only, indicating a difference in the primary diagnosis of breast cancer.

		All Observers	
		Positive	Negative
All Observers	Positive	38	1
	Negative	1	17
Overall Percent Agreement (95% CI)		96.49% (87.89-99.57)	
Positive Percent Agreement (95% CI)		97.44% (86.52-99.94)	
Negative Percent Agreement (95% CI)		94.44% (72.71-99.86)	

13 REPRODUCIBILITY RESULTS (whole tissue section testing)

Based on the results obtained in precision testing using TMA material above, further reproducibility testing using whole tissue sections on the Bond III .

13.1 Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (Test Device)

Precision results for the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the Bond III are shown below.

13.1.1 Inter-Site Reproducibility

Inter-site reproducibility testing of the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ was evaluated at 3 investigational sites (Sites A, B and C) on whole tissue sections. The test cohort consisted of 18 cases. Testing was performed over a span of 5 non-consecutive days (Day1, Day3, Day 5), with each site staining a full set of cases on each day. This provided 9 replicates of 18 cases.

Results for Inter-Site reproducibility are presented by overall (3 sites combined), single site and site to site.

Negative percent agreement, positive agreement and overall agreement for each site has been reported.

Overall (3 sites combined)

		Positive	Negative
PA0151 (RTU) Bond III	Positive	96	6
	Negative	3	57
Overall Percent Agreement (95% CI)		94.44% (89.72-97.43)	
Positive Percent Agreement (95% CI)		96.97% (91.40-99.37)	
Negative Percent Agreement (95% CI)		90.48% (80.41-96.42)	

Site A Results

		Positive	Negative
PA0151 (RTU) Bond III	Positive	35	1
	Negative	1	17
Overall Percent Agreement (95% CI)		96.30% (87.25-99.55)	
Positive Percent Agreement (95% CI)		97.22% (85.47-99.93)	
Negative Percent Agreement (95% CI)		94.44% (72.71-99.86)	

Site B Results

		Positive	Negative
PA0151 (RTU) Bond III	Positive	33	1
	Negative	0	20
Overall Percent Agreement (95% CI)		98.15% (90.11-99.95)	
Positive Percent Agreement (95% CI)		100% (91.32-100)	
Negative Percent Agreement (95% CI)		95.24% (76.18-99.88)	

Site C Results

		Positive	Negative
PA0151 (RTU) Bond III	Positive	32	0
	Negative	1	21
Overall Percent Agreement (95% CI)		98.15% (90.11-99.95)	
Positive Percent Agreement (95% CI)		96.97% (84.24-99.92)	
Negative Percent Agreement (95% CI)		100% (86.71-100)	

Site A versus Site B

		Site A	
		Positive	Negative
Site B	Positive	32	2
	Negative	4	16
Overall Percent Agreement (95% CI)		88.89% (77.37-95.81)	
Positive Percent Agreement (95% CI)		88.89% (73.94-96.89)	
Negative Percent Agreement (95% CI)		88.89% (65.29-98.62)	

Site A versus Site C

		Site A	
		Positive	Negative
Site C	Positive	32	0
	Negative	4	18
Overall Percent Agreement (95% CI)		92.59% (82.11-97.94)	
Positive Percent Agreement (95% CI)		88.89% (73.94-96.89)	
Negative Percent Agreement (95% CI)		100% (84.67-100)	

Site B versus Site C

		Site B	
		Positive	Negative
Site C	Positive	31	1
	Negative	3	19
Overall Percent Agreement (95% CI)		92.59% (82.11-97.94)	
Positive Percent Agreement (95% CI)		91.18% (76.32-98.14)	
Negative Percent Agreement (95% CI)		95.00% (75.13-99.87)	

13.1.2 Lot to Lot Precision Study

Lot to lot reproducibility testing of the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ was evaluated at a single investigational site on whole tissue sections. The test cohort consisted of 18 cases. Testing was conducted using three (3) independently manufactured reagent lots (Lot 1 = 21825; Lot 2 = 21866; Lot 3 = 21867). This format provided 3 replicates of each slide (one for each reagent lot).

		Positive	Negative
PA0151 (RTU) Bond III	Positive	32	1
	Negative	1	20
Overall Percent Agreement (95% CI)		96.30% (87.25-99.55)	
Positive Percent Agreement (95% CI)		96.97% (84.24-99.92)	
Negative Percent Agreement (95% CI)		95.24% (76.18-99.88)	

13.2 Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (Test Device)

Precision results for the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ on the Bond III are shown below.

13.2.1 Inter-Site Reproducibility

Inter-site reproducibility testing of the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ was evaluated at 3 investigational sites (Sites A, B and C) on whole tissue sections. The test cohort consisted of 18 cases. Testing was performed over a span of 5 non-consecutive days (Day1, Day3, Day 5), with each site staining a full set of cases on each day. This provided 9 replicates of 18 cases.

Results for Inter-Site reproducibility are presented by overall (3 sites combined), single site and site to site.

Negative percent agreement, positive agreement and overall agreement for each site has been reported.

Overall (3 sites combined)

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	111	0
	Negative	6	45
Overall Percent Agreement (95% CI)		96.30% (92.11-98.63)	
Positive Percent Agreement (95% CI)		94.87% (89.17-98.10)	
Negative Percent Agreement (95% CI)		100% (93.56-100)	

Site A Results

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	39	0
	Negative	0	15
Overall Percent Agreement (95% CI)		100% (94.60-100)	
Positive Percent Agreement (95% CI)		100% (92.61-100)	
Negative Percent Agreement (95% CI)		100% (81.90-100)	

Site B Results

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	35	1
	Negative	1	17
Overall Percent Agreement (95% CI)		96.30% (87.25-99.55)	
Positive Percent Agreement (95% CI)		97.22% (85.47-99.93)	
Negative Percent Agreement (95% CI)		94.44% (72.71-99.86)	

Site C Results

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	35	1
	Negative	1	17
Overall Percent Agreement (95% CI)		96.30% (87.25-99.55)	
Positive Percent Agreement (95% CI)		97.22% (85.47-99.93)	
Negative Percent Agreement (95% CI)		94.44% (72.71-99.86)	

Site A versus Site B

		Site A	
		Positive	Negative
Site B	Positive	36	0
	Negative	3	15
Overall Percent Agreement (95% CI)		94.44% (84.61-98.84)	
Positive Percent Agreement (95% CI)		92.31% (79.13-98.38)	
Negative Percent Agreement (95% CI)		100% (81.90-100)	

Site A versus Site C

		Site A	
		Positive	Negative
Site C	Positive	36	0
	Negative	3	15
Overall Percent Agreement (95% CI)		94.44% (84.61-98.84)	
Positive Percent Agreement (95% CI)		92.31% (79.13-98.38)	
Negative Percent Agreement (95% CI)		100% (81.90-100)	

Site B versus Site C

		Site B	
		Positive	Negative
Site C	Positive	34	2
	Negative	2	16
Overall Percent Agreement (95% CI)		92.59% (82.11-97.94)	
Positive Percent Agreement (95% CI)		94.44% (81.34-99.32)	
Negative Percent Agreement (95% CI)		88.89% (65.29-98.62)	

13.2.2 Lot to Lot Precision Study

Lot to lot reproducibility testing of the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ was evaluated at a single investigational site on whole tissue sections. The test cohort consisted of 18 cases. Testing was conducted using three (3) independently manufactured reagent lots (Lot 1 = 6021831; Lot 2 = 6021836; Lot 3 = 6021857). This format provided 3 replicates of each slide (one for each reagent lot).

		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	32	0
	Negative	1	21
Overall Percent Agreement (95% CI)		98.15% (90.11-99.95)	
Positive Percent Agreement (95% CI)		96.97% (84.24-99.92)	
Negative Percent Agreement (95% CI)		100% (86.71-100)	

13.3 Precision Summary

In addition to the evaluation method and in keeping with standard pathological practice, all aspects of staining quality were assessed and reported for each case, i.e., staining intensity, proportion of cells staining, background staining, tissue morphology, staining of benign ductal cells, stromal cells, endothelial cells and lymphocytes.

Cases for reproducibility studies were selected with the following estrogen receptor expression; 6 negative, 6 around cut off (1-10% tumour staining; Allred proportion score of 2) and 6 positive cases (>10% tumour staining). Reproducibility studies have shown that for cases around the cut-off (1-10% tumour staining), a single pathologist has reproducibly estimated a similar percentage of cells staining between the three replicate slides. When all three pathologist percentage of cells staining scores are compared, it has shown that some variation in the number of cells estimated exists. This shows the limitations of a subjective method based on estimation alone.

In addition, small differences in the percentage of cells stained and then estimated by the same pathologist may be due to tumour heterogeneity. Multiple sections from each tissue block are required to perform reproducibility studies, with this process resulting in different cells being examined between each section. Small foci of tumour will invariably cut in/out of the tissue section and result in differences in interpretation. This phenomenon has been widely published and the concept is most effectively demonstrated by the micro-dissection of sentinel node tumour micro-metastases analysis (Weaver 2010).

Based on acceptance and approval of the Test Device **Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the Bond III**, this device is now considered the **Reference Standard Test** for the further Inter-Platform Comparison Studies.

14 Inter-Platform Comparison Study

An inter-platform comparison study was performed at three independent US based testing facilities. Comparative data between the reference standard device **Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ on the Bond III** and **Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, NovocastraTM on the Bond III.**

Blinding and randomization was used throughout the evaluation to reduce bias. Screeners were blinded to the test used.

The sample types (specimens) used in clinical evaluation were whole tissue sections, formalin fixed, and paraffin embedded invasive breast cancer cases. The specimens utilized were obtained from clinical archives, representative of the intended population, samples appropriately handled in the clinical environment, and met all local and national institutional review board (IRB) ethical approval for use in clinical evaluation studies.

Positive procedural controls were performed to validate assay performance. Procedural controls were not used for statistical analysis.

Negative controls were performed on the same tissues as the test article. The negative controls were not used for statistical analysis.

14.1 Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ on the Bond III (Test Device)

Inter-Platform comparison study results for Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ on the Bond III compared against the reference standard test using the primary evaluation method are shown below.

		Reference Standard Test	
		Positive	Negative
NCL-L-ER-6F11 (Concentrate) Bond III	Positive	241	6
	Negative	4	55
Overall Percent Agreement (95% CI)		96.73% (94.07-98.42%)	
Positive Percent Agreement (95% CI)		98.37% (95.87-99.55)	
Negative Percent Agreement (95% CI)		90.16% (79.81-96.30)	

Based on the results, all documented evidence supports substantial equivalence between the both the concentrated and RTU formats of ER 6F11 on the Bond III.

14.2 Inter-Platform Comparison Study Summary

The total cohort included for the Inter-Platform comparison study was three hundred and six (306) invasive breast cancer specimens.

A requirement set by FDA for the cohort to include a minimum of 10% of test cases that express estrogen receptor around the cut off (1-10%), when assessed by the reference standard device, was not met. The total overall percentage of cases in this category was 7.5% (23/306). Assessment of an additional 452 cases was undertaken in an attempt to enrich the cohort to 10%.

Recent publications (Hicks DH, Zhang Z, *et al*), in which 1700 consecutive invasive breast cancer patients were assessed for ER status, indicated that only 2% of cases expressed estrogen in the 1-10% range. This indicates that these tumours are not common and that the 7.5% of cases assessed within this cohort is representative of almost 4 times the normal population.

Additionally, differences in the percentage of cells stained and then estimated by the same pathologist between test devices may be due to tumour heterogeneity. Multiple sections from each tissue block are required to perform comparison studies, with this process resulting in different cells being examined between each section. Foci of tumour will invariably cut in/out of the tissue section and result in differences in interpretation. This phenomenon has been widely

published and the concept is most effectively demonstrated by the micro-dissection of sentinel node tumour micro-metastases analysis (Weaver 2010).

In addition to the evaluation method and in keeping with standard pathological practice, all aspects of staining quality were assessed and reported for each case, i.e., staining intensity, proportion of cells staining, background staining, tissue morphology, staining of benign ductal cells, stromal cells, endothelial cells and lymphocytes.

15 Stability Summary

Stability studies were performed using three independently manufactured lots of Estrogen Receptor Clone 6F11 for the different formats of the product listed below.

- Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ – 7 mL PA0151
- Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ – 30 mL PA0009
- Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ – 1 mL NCL-L-ER-6F11

Testing was performed on breast cancer tissue sections that expressed estrogen receptor at a high (3), medium (2), low (1) and negative (0) intensity (Intensity Score) and on normal breast tissue.

Acceptance criteria is based on; Test case passes if the Intensity Score and the Proportion Score (≥ 1% of tumour cell nuclei staining = Positive; < 1% of tumour cell nuclei staining = Negative) are identical for each of the test estrogen expressive breast cancer cases used when compared to a control score. A test will fail if this criteria is not met.

Stability claims to date, based on accelerated testing for the three listed product formats above is:

- 18 months (545 days) from the point of manufacture when stored at 2 – 8 °C.

Real time stability data (18 months) will be tested for the Ready-to-Use formats of the product on the 20 May 2014. Real time stability data (18 months) will be tested for the Liquid concentrate format of the product on the 17 June 2014.

Transportation stress testing and In-Use stability testing has additionally been conducted and shown to be acceptable.

The table below shows a summary of the stability testing to date and indicates time point for continued testing.

Stability Summary

ER Formats	Tissue	Number of Batches Tested	Scoring	Acceptance Criteria	Results	Next Real Time Test Point (Date & Timepoint)
Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™, 7mL PA0151	Variable ER expression (IS) Breast Carcinoma	3	Scoring based on: Nuclear intensity score (0 = Negative; 1 = Weak; 2 = Intermediate; 3 = Strong) & Proportion score (> 1% of tumour cell nuclei staining = Positive; < 1% of tumour cell nuclei staining = Negative)	A test passes if the Intensity Score (IS) and the Proportion Score (PS) are identical for each of the variable ER expressive breast carcinoma cores on the TMA when compared to the Control score. (PS score is not applicable for the normal breast core)	Report issued with 18 Months shelf life via accelerated testing	20/05/2014 - 12 Months
	High (Nuclear Intensity Score 3)					
	Medium (Nuclear Intensity Score 2)					
	Low ((Nuclear Intensity Score1)					
	Negative (Nuclear Intensity Score 0)					
	Normal breast					
Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™, 30mL PA0009	Variable ER expression Breast Carcinoma	3			Report issued with 18 Months shelf life via accelerated testing	20/05/2014 - 12 Months
	High (Nuclear Intensity Score 3)					
	Medium (Nuclear Intensity Score 2)					
	Low ((Nuclear Intensity Score1)					
	Negative (Nuclear Intensity Score 0)					
	Normal breast					
Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™, NCL-L-ER-6F11 (1mL) for use on Bond	Variable ER expression Breast Carcinoma	3			Report issued with 18 Months shelf life via accelerated testing	17/06/2014 - 12 Months
	High (Nuclear Intensity Score 3)					
	Medium (Nuclear Intensity Score 2)					
	Low ((Nuclear Intensity Score1)					
	Negative (Nuclear Intensity Score 0)					
	Normal breast					

16 Conclusions

Estrogen Receptor Clone 6F11, was determined to have excellent correlation for the determination of endocrine treatment response in breast cancer based on data performed in an independent Clinical Outcome Study (Calgary Cohort). This study also showed clinical correlation of the Estrogen Receptor Clone 6F11 with previously 510(k) approved devices (Ventana K110215; Dako K042884).

Additional inter-platform validation has shown excellent correlation between the different formats and the reference standard device.

This 510(k) submission describes the antibody as being available in the various formats. These formats include:

- Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ (PA0151, PA0009), which is optimized for use on the Leica Biosystems Bond™ automated slide staining system (Bond III).
- Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ (NCL-L-ER-6F11, 1mL) for use on the Leica Biosystems Bond™ automated slide staining system (Bond III)

Reproducibility testing has additionally shown that all formats of the Estrogen Receptor Clone 6F11 perform adequately.

Therefore, based on the information provided in the premarket notification, Leica Newcastle concludes that both the Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ and the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ of estrogen receptor on the Bond III demonstrate similar performance to the predicate device K060227 for the indication for use, device design, material, operational principles and intended use.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

May 19, 2014

Leica Biosystems Richmond, Ltd
Barbara Ann Conway-Myers, Ph.D.
Senior Regulatory Affairs Specialist
5205 Route 12
Richmond, IL 60071

Re: K122556
Trade/Device Name: Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™
Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™
Regulation Number: 21 CFR 864.1860
Regulation Name: Immunohistochemistry Reagents and Kits
Regulatory Class: II
Product Code: MYA
Dated: April 8, 2014
Received: April 10, 2014

Dear Dr. Conway-Myers:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Reena Philip -S

Reena Philip, Ph.D.
Director
Division of Molecular Genetics and Pathology
Office of *In Vitro* Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K122556

Device Name
Estrogen Receptor Clone 6F11 (ER 6F11)
- Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™;
- Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™

Indications for Use *(Describe)*
Estrogen Receptor Clone 6F11 (ER 6F11) Mouse Monoclonal antibody is intended for laboratory use to qualitatively identify estrogen receptor (ER) antigen in sections of formalin fixed, paraffin embedded breast cancer tissue by immunohistochemistry methods. Estrogen Receptor Clone 6F11 specifically binds to the ER antigen located in the nucleus of ER positive normal and neoplastic cells.

Estrogen Receptor Clone 6F11 is indicated as an aid in the management, prognosis and predication of therapy outcome of breast cancer. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by qualified pathologist.

Estrogen Receptor Clone 6F11 Ready-to-Use Primary Antibody for Bond™ and the Estrogen Receptor Clone 6F11 Liquid Concentrate Primary Antibody, Novocastra™ are optimized for use on the Leica Biosystems Bond III staining platform using the Bond Polymer Refine Detection Kit.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

Yun-fu Hu -S

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